

Full Length Research Paper

Evaluation of the simultaneous effects of a heat stabilized starter concentration and the duration of fermentation on the quality of the opaque sorghum beer

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This study evaluates the simultaneous effect of dried starter concentration and fermentation duration on the quality characteristics of the African opaque sorghum beer using response surface methodology. The aim was to improve the beer quality and to optimize its fermentation process. Results show that the granule starter concentration and the fermentation duration have significant effects on the dry matter, refractive index, titrable acidity, glucose, raffinose and fructose contents of the beer. The lactic acid bacteria, yeasts and total mesophilic aerobic bacteria counts were significantly modified as a result of these fermentation parameters. The pH was only affected by the fermentation duration. The optimum beer quality could be reached between 10 and 15 h at starter concentration in the interval of 100 to 120 g/L. The use of the dried starter granules revealed to be an efficient alternative to produce sorghum beer with stable quality at a shorter fermentation time.

Key words: starter, kpete-kpete, sorghum beer, tchoukoutou.

INTRODUCTION

Opaque sorghum beers significantly contribute to the diet of millions of people in Africa due to their relatively low alcoholic content and high dry matter and nutrients concentration (Novellie and De Schaepdrijve, 1986). The beers are known as *tchoukoutou* in Benin (Kayodé et al., 2005), *dolo* in Burkina-Faso (Dicko et al., 2006), *burukutu* or *otika* in Nigeria (Odunfa, 1985), *bili bili* in Tchad (Maoura et al., 2005), *Kaffir* in South Africa (Novellie and De Schaepdrijver, 1986), *doro* or *chibuku* in Zimbabwe

(Chamunorwa et al., 2002) and *ikigage* in Rwanda (Lyumugabe et al., 2010). *Tchoukoutou*, the Benin opaque sorghum beer, is produced from guinea corn (*Sorghum bicolor*) by women using various processes. In general, as in the conventional lager beer process, the manufacturing process consists of three main phases: malting, mashing and fermentation. Grains of sorghum are soaked in water overnight (9 to 12 h), germinated (72 to 85 h), sun dried (7 to 15 h), ground in a disc mill, mixed

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with water, decanted and divided into slurry and supernatant. The slurry is mashed under gradual heating until the boiling point is reached after 2 h, mixed with supernatant and allowed to ferment overnight, then filtered, boiled (6 to 9 h), cooled, and inoculated with a starter called *kpete-kpete*, which is generally harvested from the bottom of a previous fermenting beer (resulting from 13 to 14 h overnight fermentation). The fermentation step is a critical step in the process, and its success depends on the accurate knowledge of the processor in terms of the starter handling. Several researches reported that the microorganisms contained in the traditional starters of African opaque beers mainly consist of yeasts and lactic acid bacteria (LAB) (Van der Aa Kühle et al., 2001; Demuyakor and Ohta, 1991; Sefa-Deheh et al., 1999; Sanni and Lönner, 1993). The microorganisms are kept alive by replacing the supernatant on a daily basis. The preservation of such starter is a tedious and a risky business since it is common that the starter loses its fermenting properties and therefore fails to make the beer effervescent, as a result of the death of the involved microorganisms.

Preliminary data demonstrated that rural and urban women's groups in the processing chain of opaque sorghum beers derive a direct benefit from increased marketing opportunities (Kayodé et al., 2007). Thus, innovations in the traditional brewing technology and the product quality could significantly improve the income and livelihood of rural households involved in this activity. Recently, Kayodé et al. (2012) defined a granule starter for the fermentation of opaque sorghum beer; but still, the effective dose and the fermentation duration for this granule are not determined.

The aim of the present study was to determine the optimum doses of the granule starter for the fermentation of the African opaque sorghum beers. More specifically, the objective was to evaluate the effect of the starter concentration and the fermentation duration on several beer quality in determining various factors such as dry matter, refractive index, pH, titratable acidity, ethanol, glucose, raffinose, fructose, maltose, yeast, LAB and total mesophilic aerobic bacteria counts of the fermenting beer. It is quite likely that these factors are interdependent. However, interactions between factors cannot be detected using the one-factor-at-a-time approach (Giovani, 1983). Therefore, we used the response surface method in applying a central composite design.

MATERIALS AND METHODS

Production of the granule starter and wort

The granule starters were produced using a tannin-free sorghum variety, yeasts and lactic acid bacteria harvested from the traditional starter of opaque sorghum beer. The processing procedure was according to the method described by Kayodé et al. (2012). Sorghum wort was produced according to the traditional brewing

practices, as follows: sorghum grain were soaked overnight (10 h), germinated (72 h), sun to dried (12 h), ground in a disc mill, mashed in water by gradually heating until the boiling point was reached after 2 h, soured during an overnight rest, filtered, boiled (8 h) and cooled.

Experimental design

Response surface methodology is a statistical method that uses quantitative data derived from an appropriate experimental design, with quantitative factors use to estimate the relationship between a response and the factors in order to optimize processes or products (Giovani, 1983). In this study, an orthogonal rotatable central composite design (Montgomery, 2001) for $K = 2$ factors was used, to estimate the simultaneous effect of two process variables on physico-chemical and microbiological characteristics of sorghum fermenting beer in a quadratic function. The variables (factors) were the starter concentration (0-150 g/L), and the fermentation duration (0 to 24 h). In this experimental design, dry matter, refractive index, pH, titratable acidity, ethanol, glucose, raffinose, fructose, maltose, yeast, LAB and total mesophilic aerobic bacteria counts are considered as the responses. The design generated 14 observations which are distributed as follows: 4 kernel points, 4 star points and 6 replications at the central point. The design matrix and variable combinations are presented in Table 1.

Experimental fermentation

Sorghum wort (14 L) was inoculated with the granule starter at concentrations varying between 0 and 150 g/L. The inoculated wort was allowed to ferment at room temperature for an indicated time as specified in the experimental design (Table 1). When the predefined times were reached at a given starter concentration, samples were withdrawn, divided into two parts. The first part was immediately analyzed for microbiological characteristic while the second part was kept at -4°C for physicochemical analyses.

Counts of microorganisms

Total counts of mesophilic aerobic bacteria, LAB, yeasts and molds were enumerated according to the method described by Nout et al. (1987). Duplicate samples of stabilized starter (10 g) were diluted in 90 ml sterile peptone physiological saline solution (5 g peptone, 8.5g NaCl, and 1000 mL distilled water, pH = 7.0) and homogenised with a Stomacher lab-blender (type 400, London, UK). Decimal dilutions were plated. Total mesophilic aerobic bacteria counts were determined on plate count agar (PCA, oxoid, CM 325, Hampshire, England) after incubation at 30°C for 72 h. Viable counts of LAB were determined on the Man, Rogosa and Sharpe agar (MRSA, CM 361, Oxoid, Hampshire, England) containing 0.1% (w/v) natamycin (Delvocid, DSM, The Netherlands) after incubation at 30°C for 72 h in anaerobic jar (Anaerocult A, Merck KGaA, Germany). Viable yeasts were determined on Oxytetracyclin glucose yeast extract agar (OGYA, Oxoid CM 0545, Basingstoke, Hampshire, England) after incubation at 25°C for 72 h.

Physico-chemical analysis

Titratable acidity and pH were determined as described by Nout et al. (1989). Dry matter was determined according to the American Association of Cereal Chemists (AACC) Approved Methods (AACC, 1984). The refractive index was measured using a refractometer (Sopelem 9596, France).

Table 1. Matrix of the model and combination of variables

Numbers	Level codes		Level of the variable	
	Quantity of starter	Fermentation duration	Quantity of starter (g/ L of wort)	Fermentation duration (h)
1	0	0	75	12
2	0	0	75	12
3	0	0	75	12
4	0	0	75	12
5	0	0	75	12
6	0	0	75	12
7	-1	-1	29.92	4.79
8	+1	-1	120.07	4.79
9	-1	+1	29.92	19.21
10	+1	+1	120.07	19.21
11	0	-α	75	0
12	0	+α	75	24
13	-α	0	0	12
14	+α	0	150	12

HPLC analysis of sugars and alcohol

Ethanol and soluble sugars were determined following the method developed by Mestres and Rouau (1997) using the column Aminex HPX87H-Biorad (Hercules, USA) thermostated at 37°C. Elution was done with sulfuric acid 5 mM at a flow rate of 0.6 mL/min. Detection was at 210 nm. Analyses were performed in triplicate.

Statistical analysis

Data were analyzed using the Minitab 14 statistical program. A second order polynomial model was proposed to establish the relationship between the responses (Y) and the variables (X) as follows:

$$Y = b_0 + b_1X_1 + b_2X_2 + b_3X_1^2 + b_4X_2^2 + b_5X_1X_2$$

Where, b_0 is constant, b_1 and b_2 are linear effect coefficients, b_3 and b_4 are quadratic effect coefficients, and b_5 is an interaction affect coefficient. The fitted polynomial equations were expressed in a 3D response surface in which the response is presented on the vertical axis and two factors at the two horizontal perpendicular axes.

RESULTS AND DISCUSSION

Effects of starter concentration and fermentation duration on dry matter and refractive index

The response values for the different treatments are presented in Table 2. The linear regression coefficients estimated are presented in Table 3. After the different treatments applied, the dry matter and the refractive index ranged between 3.42 and 15.15%, 9.50 and 15.00 (Table 2), respectively. The analysis of variance showed that the starter concentration (X_1) and the fermentation duration (X_2) significantly affect ($P < 0.001$) the dry matter content and the refractive index of the wort. Particularly,

the linear effects of X_1 and X_2 on the dry matter content of wort are significant whereas the refractive index of the wort is significantly affected both by the linear and the quadratic effects of these factors (Table 3). Figure 1b shows the trend in refractive index as function of the starter concentration, the fermentation duration and their mutual interaction. Increase in the starter concentration and the fermentation duration results in a decrease in the refractive index values. This trend could be explained by the fact that the increase of starter concentration raised the level of microorganisms and then facilitated the degradation of soluble sugars. This degradation was marked for starter concentration in the range between 0 and 120 g/L and for the fermentation duration between 0 and 10 h. In opposite, we observed that when the starter concentration increased, the dry matter content of the wort also increased. Indeed, the high value in dry matter content (92.5 %) of granule starter used would have significantly contributed to the beer dry matter content.

Interestingly, one of the most important characteristic of African sorghum beers is their relatively high dry matter content which range between 5 to 13 g/100 ml (Agu and Palmer, 1998; Briggs et al., 2004). In the present experiment, it could be seen from the response surface plot that such value of dry matter content could be achieved at starter concentration between 100 and 120 g/L and fermentation duration at an interval of 20 and 22 h.

Effects on pH and titratable acidity

Acidity is one of the most important quality criteria to measure the acceptability as well as the stability and the conservation of the fermented foods (Kayodé et al., 2005). The acidity of the beer is measured through pH

Table 2. Response of the model for the physicochemical and microbiological characteristics of the wort.

Code	Dry matter (%)	Refractive index	pH	Titrateable acidity (% acide lactique)	Ethanol (%)	Glucose (mg/100g)	Raffinose (mg/100g)	Fructose (mg/100g)	Maltose (mg/100g)	Yeast ($\log_{10}$ CFU ml^{-1})	Lactic acid bacteria ($\log_{10}$ CFU ml^{-1})	Total count ($\log_{10}$ CFU ml^{-1})
1	8.78	9.50	3.98	1.20	2.45	0.66	0.48	0.00	0.00	8.24	8.86	9.98
2	8.18	9.80	3.99	1.30	2.45	0.62	0.48	0.00	0.00	8.07	8.61	9.79
3	8.73	9.80	3.99	1.30	2.45	0.60	0.36	0.00	0.00	8.91	8.2	9.88
4	8.46	9.70	3.98	1.40	2.45	0.57	0.44	0.00	0.00	8.21	8.86	9.95
5	8.26	9.80	3.97	1.40	2.45	0.77	0.50	0.00	0.00	8.92	8.19	9.91
6	8.74	9.80	3.91	1.30	2.45	0.74	0.62	0.00	0.00	8.93	8.89	9.96
7	6.19	13.80	4.21	1.30	2.45	3.36	2.05	0.40	8.92	7.18	6.91	7.92
8	11.28	12.00	4.15	0.80	1.65	2.40	1.41	0.15	4.07	7.72	7.56	7.82
9	5.07	9.80	3.77	2.40	2.31	0.35	0.51	0.00	0.00	8.13	8.11	8.36
10	9.41	10.00	4.19	1.40	2.25	0.38	0.28	0.00	0.00	8.21	8.08	8.65
11	12.58	15.00	4.18	0.90	1.06	3.10	1.92	0.40	0.00	6.22	6.08	6.32
12	6.70	10.00	3.73	2.10	2.81	0.42	0.41	0.00	0.00	8.43	8.38	9.76
13	3.42	14.00	4.26	2.10	0.45	2.96	2.09	0.65	0.00	6.40	7.23	7.42
14	15.15	9.80	3.84	0.90	2.57	0.60	0.34	0.00	0.00	7.80	7.84	8.26

Table 3. Values of the coefficients in the model and their significance

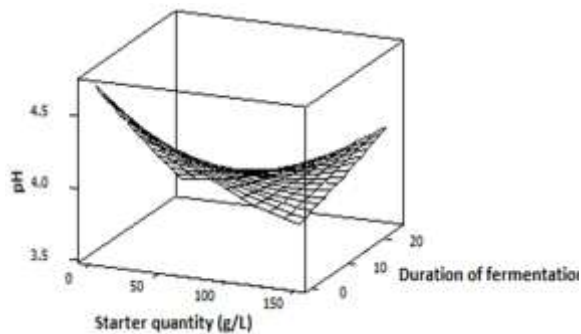
Coefficient	Dry matter (%)	Refractive index	pH	Titrateable acidity	Ethanol	Glucose	Raffinose	Fructose	Maltose	Yeast	Lactic acid bacteria	Total count
b_0	8.382***	9.720***	3.980***	1.319***	2.478***	0.677**	0.487***	-0.006	0.382	8.573***	8.595***	9.876***
b_1	5.050***	-1.498***	-0.059	-0.610***	0.465	-0.846**	-0.661***	-0.231***	-0.846	0.514*	0.285	0.277
b_2	-2.229***	-2.499***	-0.200*	0.645***	0.588	-1.65***	-0.904***	-0.213***	-2.266	0.893**	0.968***	1.220***
b_3	0.345	2.127***	0.109	0.192**	-0.858	1.174**	0.758**	0.308***	1.109	-1.369**	-1.085**	-2.174***
b_4	0.701	2.727***	0.014	0.192**	-0.433	1.154**	0.707**	0.184*	1.108	-1.144**	-1.391***	-1.974***
b_5	-0.519	1.385	0.332	-0.346**	0.512	0.686	0.282	0.169	3.357	-0.318	-0.471	0.270
R^2	86.4	93.8	39.4	97.8	35.5	89.5	90.1	86.2	0.550	76.4	86.4	86.5

b_0 : constant; b_1 and b_2 : coefficients for starter concentration; b_3 and b_4 : coefficients for duration of fermentation; and b_5 : coefficient for interaction (starter concentration x duration of fermentation). *Significant at $p < 0.05$. **Significant at $p < 0.01$. ***Significant at $p < 0.001$. Data reported in this table are the measured (fitted) values of the coefficients b_0 , b_1 , b_2 , b_3 , b_4 and b_5 , which are explained in detail in the statistical analysis section.

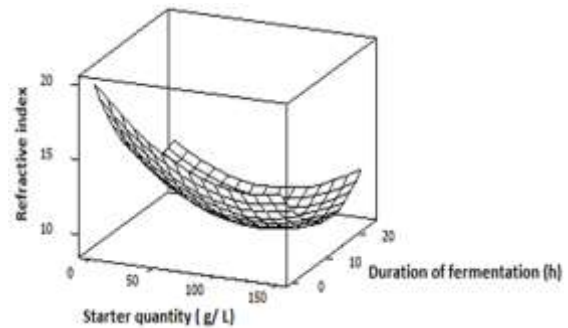
and titrateable acidity. After the different treatments applied, pH ranged between 3.73 and 4.26 whereas titrateable acidity ranged between 0.80

and 2.40 g kg^{-1} d.w (Table 2). The analysis of variance showed that, starter concentration did not significantly affect the pH but the linear effect

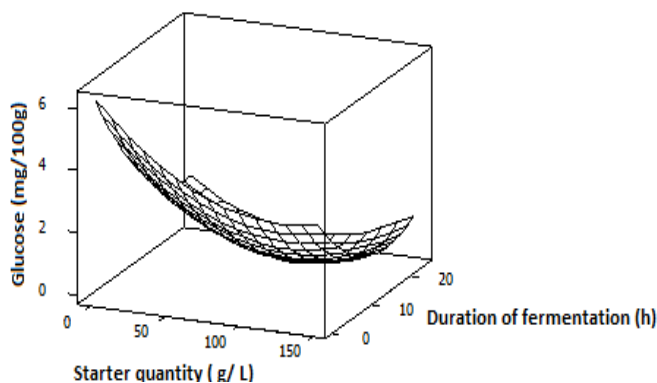
of the fermentation duration is significant ($p < 0.05$). Figure 1a shows the trends in pH of wort which functions as starter concentration, the



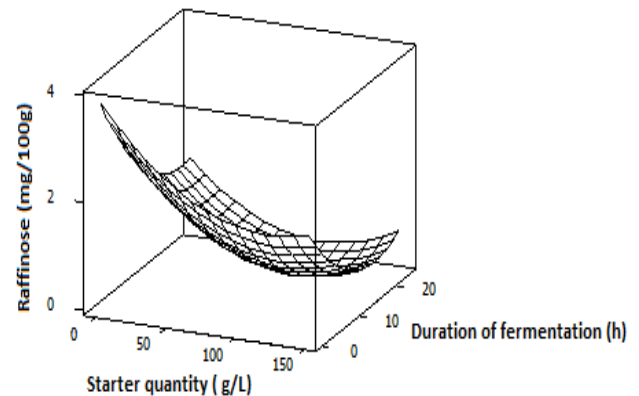
a) Effects on pH



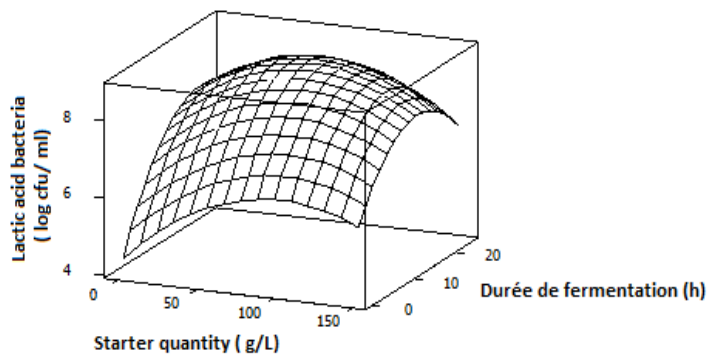
b) Effects on refractive index



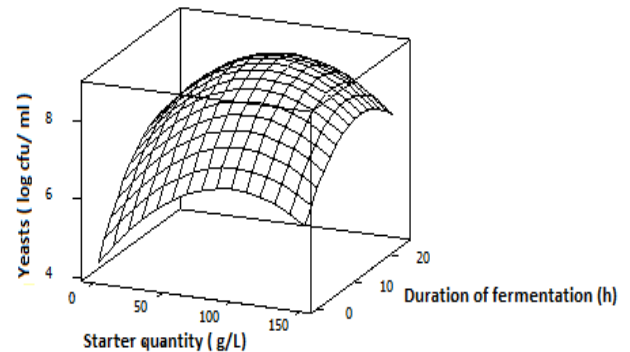
c) Effects on glucose concentration



d) Effects on raffinose concentration



e) Effects on lactic acid bacteria content



f) Effects on yeasts content

Figure 1. Response surface curves showing the effects of starter concentration (g L^{-1} starter in wort) and duration of fermentation on (a) pH, (b) refractive index, (c) glucose, (d) raffinose, (e) lactic acid bacteria and (f) yeasts content of sorghum wort.

fermentation duration and their mutual interaction. At fermentation duration > 20 h the pH was around 3.73 for starter concentrations between 120 and 150 g/L. For the titratable acidity, it was significantly ($p < 0.001$) affected by the two factors (X_1 and X_2). The linear and quadratic effects of these factors were significant on the wort titratable acidity (Table 3). The titratable acidity increased with the fermentation duration whereas it decreased when the starter concentration increases. As expected,

the fermentation duration significantly affects the pH and the titratable acidity. This effect was expressed by a decrease in the pH and an increase in the product acidity when the fermentation duration increases. Previous studies reported that the process of sorghum wort fermentation is characterized by the increase of titratable acidity and the decrease of the pH (Hounhouigan et al., 1999; Mugula et al., 2003; Annan et al., 2003) and such trend is a characteristic of cereals fermented foods.

Effects on alcohol and soluble sugars contents

Ethanol has been identified as an important metabolic compound along with the different treatments applied. Sanni and Lönner (1993) identified ethanol as an essential alcoholic compound obtained from the fermentation process during the production of local sorghum beers such as pito, burukutu, sekete and agadagidi. Ethanol concentrations of beers from the different treatments were ranged between 0.45 and 2.81% (Table 2). Studies reported that the ethanol content in various African sorghum beers range between 2 and 3% (Sanni and Lönner, 1993; Kayodé et al., 2005). The analysis of variance showed that none of the factors significantly affected the ethanol concentration. This trend is in line with the results reported by N'Gessan et al. (2008) who found that, the increase of the inoculum concentration did not affect the ethanol production during sorghum wort fermentation.

According to the Mateo et al. (2001), in such inoculums, various strains of yeasts could be in an ecological competition. In our case the yeast strains contained in the starter used were not selected. Though to the ecological competition, the effect of the starter concentration and the fermentation duration was not significant. It is necessary to isolate the main yeast strains which contained in the starter in order to assess the effects of both factors on the beer alcohol content.

The soluble sugars identified in the fermented wort were: glucose, raffinose, fructose and maltose. After the different treatments applied, the concentration of these soluble sugars ranged between: 0.35 and 3.36 mg/100 g for glucose; 0.28 and 2.09 mg/100 g for raffinose; 0.00 and 0.651 mg/100 g for fructose; and 0.00 and 8.921 mg/100 g for maltose (Table 2). The analysis of variance showed that none of the factors significantly affected the maltose content while, the starter concentration (X_1) and the fermentation duration (X_2) significantly ($P < 0.05$) affected glucose, raffinose and fructose contents. Particularly, the linear and the quadratic effects of these factors were significant ($p < 0.01$) on these sugars content (Table 3). Figure 1a and d show, respectively the evolution of glucose and raffinose contents as a function of the starter concentration, the fermentation duration and their mutual interaction. The trends were quite similar. The two factors increased while the soluble sugars contents (glucose, raffinose and fructose) decreased. This effect was significant at starter concentrations ranging from 0 to 120 g/L and at fermentation duration from 0 to 20 h. Clearly, the increase of starter concentration increases the fermentation activities in the wort. During the alcoholic fermentation process, yeasts transform these soluble sugars into ethanol (Leyral and Vierlin, 2007). After 20 h of fermentation, the increase of the starter concentration did not result in the increase of soluble sugars consumption. The main incriminated factor could be nitrogen limitation (Barre et al., 1998;

Manginot et al., 1998). It was also reported that the amount of assimilable nitrogen influences the synthesis of sugars transporters (Bisson, 1999).

Effects on microbial content

The major microorganisms involved in the fermentation of opaque sorghum beer are LAB and yeasts (Maoura et al., 2005; Lyumugabe et al., 2010). As a result of the treatments applied, the number of yeasts, lactic acid bacteria and total mesophilic aerobic bacteria varied significantly as a function of the two factors and are respectively in the range between 6.08 and 8.89 log CFU/ml; 6.22 and 8.93 log CFU/ml; and 6.32 and 9.98 log CFU/ml. The analysis of variance showed that the starter concentration and the fermentation duration significantly ($P < 0.05$) affect the different microorganisms groups. Particularly, the quadratic effects of these factors on the microbial content were very significant ($p < 0.001$). Figure 1 shows the trends in the number of microorganisms as a function of the starter concentration, the fermentation duration and their mutual interaction. High level of the starter concentration and fermentation duration results in high concentration of microorganisms in the wort. This trend is more remarkable for values in starter concentrations ranging between 0 and 50 g/L, and for fermentation duration between 0 and 10 h. This observation could be explained by the intensive fermentation rates due to the load and the faster microflora development at the highest rates of inoculation (Gotcheva et al., 2001). After 20 h of fermentation, the yeasts counts decreased. This could probably be due to the inhibitory effect of ethanol on yeasts growth. It was reported that a high content of ethanol in the wort can inhibit the growth, the viability, the metabolic activity of fermentative yeasts (Aguilera et al., 2006; Canetta et al., 2006; Hu et al., 2006; Hirasawa et al., 2007; Kitagaki et al., 2007; Lei et al., 2007; Wang et al., 2007; Watanabe et al., 2007; Wei et al., 2007).

Relationship between beer quality parameters

The Pearson correlation matrix of variables (Table 4) showed a correlation between the pH and others parameters such as: Refractive index ($r = 0.722$; $p < 0.01$), ethanol rate ($r = -0.680$; $p < 0.01$), glucose concentration ($r = 0.752$; $p < 0.01$), yeasts ($r = -0.623$; $p < 0.05$) and LAB ($r = -0.598$; $p < 0.05$). It also revealed a significant relationship between the refractive index and ethanol concentration ($r = -0.780$; $p < 0.001$), glucose concentration ($r = 0.967$; $p < 0.001$), yeast counts ($r = -0.899$; $p < 0.001$) and lactic acid bacteria content of the wort ($r = -0.913$; $p < 0.001$). The pH was negatively correlated with the ethanol content, yeasts and the LAB. This is in accordance with the fermentation characteristics

Table 4. Pearson correlation matrix of variables.

Parameter	Refractive index	pH	Ethanol	Glucose	Yeasts
pH	0.722**				
Ethanol	-0.780***	-0.680**			
Glucose	0.967***	0.752**	-0.717**		
Yeasts	-0.899***	-0.623*	0.798***	-0.829***	
Bacteria	-0.913***	-0.598*	0.662**	-0.848***	0.836***

*Significant at 5%; **significant at 1%; ***significant at 1%.

of the African traditional beer which is a mixed fermentation that is lactic fermentation and alcoholic fermentation (Valyasevi and Rolle, 2002). During the lactic fermentation the increase in LAB content results in the production of lactic acid which lowers the pH and favours the yeasts growth. The ethanol content was positively correlated with the yeasts and LAB counts.

The alcoholic fermentation is characterized by the increase in yeasts which transform soluble sugars into ethanol (N'Guessan et al., 2008). That could also explain the negative correlation between glucose concentration and ethanol ($r = -0.717$; $p < 0.01$). Yeasts are positively correlated with LAB ($r = 0.836$; $p < 0.001$). A symbiotic relationship between yeasts and LAB was previously reported (Nout, 1991; Savova and Nikolova, 2002). LAB create an acid environment which are favorable to yeasts growth (Yao et al., 2009) and produce vitamins to increase other factors, such as amino acids, to aid the growth of LAB (Lyumugabe et al., 2010).

Conclusion

The use of dried starter granules derived from the traditional starter brought significant improvements in the beer quality at relatively short fermentation duration. The application of the response surface methodology revealed the linear effects of the processing parameters as well as their mutual interactions. We recommend the use of the granule starter in the fermentation of African opaque sorghum beers for the production of stable beer at shorter fermentation duration.

Conflict of interests

The authors have not declared any conflict of interests.

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